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Carbon Metabolism and Bioplastic Production by Halophilic Archaea

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1 Introduction

Haloarchaea represents a distinct group of Archaea that typically inhabits hypersaline environments, such as salt lakes and sea salterns. They are easy to culture and many haloarchaea are genetically tractable, hence they are excellent model systems for research of archaeal genetics, metabolism, and environmental adaptation. Furthermore, haloarchaea are capable of biosynthesis of extracellular polysaccharide, polyhydroxyalkanoates (PHA), bacteriorhodopsin, carotenoids and halophilic enzymes, etc., thus also have considerable biotechnological potentials. In the past a few years, we have systematically studied the carbon metabolism and PHA biosynthesis in haloarchaea, mainly with *Haloferax mediterranei* as a promising haloarchaeal cell factory (Fig.1).

2 Results and discussion

The *H. mediterranei* is a metabolically-versatile haloarchaeon, which is capable of biosynthesis of poly (3-hydroxybutyrate-*co*-3-hydroxyvalerate) (PHBV), a desirable bioplastic among PHAs, from unrelated carbon sources including some inexpensive raw materials, like starch, extruded rice bran and whey. Therefore, we have shown great interests in the carbon metabolism of this haloarchaeon, including sugar transportation (Cai *et al.*, 2014), chitin catabolism (Hou *et al.*, 2013b), and especially, the PHBV biosynthesis pathway (Han et al., 2013) as well as the genome-wide regulation of PHA production (Liu *et al.*, 2013). These studies were greatly facilitated by the availability of the genome sequences of the haloarchaea (Han *et al.*, 2012; Liu *et al.*, 2011b) and their highly efficient gene knockout systems (Liu *et al.*, 2011a).

Briefly, we have demonstrated that *H. mediterranei* biosynthesizes PHBV from acetyl-CoA and propionyl-CoA *via* a three-step process catalyzed by β-ketothiolase (PhaA/BktB), β-ketoacyl-CoA reductase (PhaB1/PhaB2), and PHA synthase (PhaEC) sequentially, with novel enzymes we identified for the first time in haloarchaea (Table 1). Notably, the two β-ketothiolases (PhaA and BktB) with different substrate specificities are distinct in both subunit composition and catalytic residues from bacterial PhaA/BktB (Hou *et al.*, 2013a). Two β-ketoacyl-CoA reductases, designated as PhaB1 and PhaB2 respectively, have also been identified to supply monomers for PHBV synthesis, of which PhaB2 is the major reductase (Feng *et al.*, 2010). As for the PHA

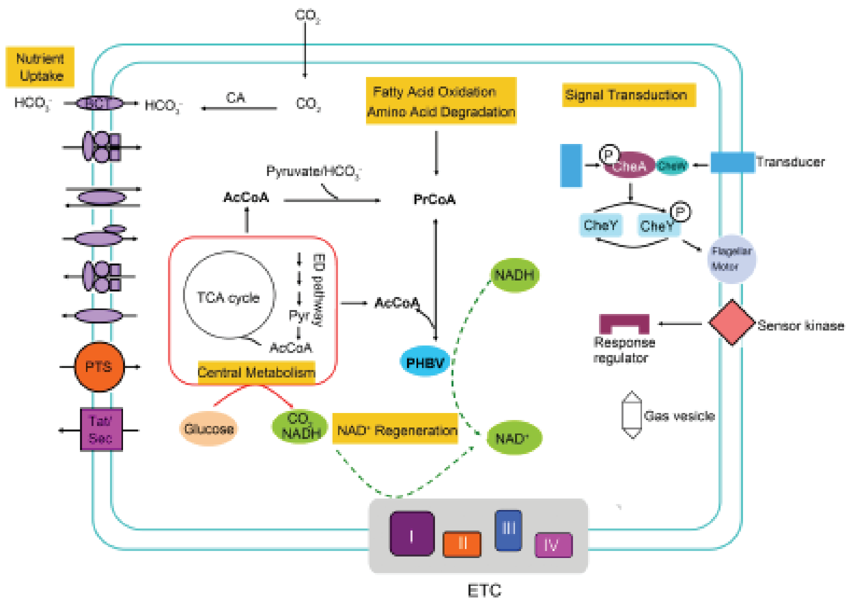

Fig. 1. An integrated view of carbon metabolism and PHBV biosynthesis in *Haloferax mediterranei*.

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Table 1 Genes involved in PHBV synthesis by H. mediterranei

Enzyme/Protein	Gene	HFX ID	Reference
PhaA and BktB	*phaA*α	HFX_1023	Hou *et al*., 2013a
	*phaA*β	HFX_1022	
	*bktB*α	HFX_6004	
	*bktB*β	HFX_6003	
PhaB1 and PhaB2	*phaB1*	HFX_1281	Han *et al*., 2009;
	phaB2	HFX_5215	Feng *et al*., 2010
PHA synthase	*phaE*	HFX_5220	Han *et al*., 2007;
	phaC	HFX_5221	Lu *et al*., 2008;
	phaC1	HFX_2678	Han *et al*., 2010a;
	phaC2	HFX_6061	Han *et al*., 2010b
	phaC3	HFX_6429	
Phasin	*phaP*	HFX_5219	Cai *et al*., 2012

synthase, a novel archaeal subtype (IIIA) of type III PHA synthase represented by the one composed of $PhaE_{Hme}$ and $PhaC_{Hme}$ has been proposed, which is distinguished from bacterial subtype (IIIB) and widely distributed in haloarchaea (Lu *et al*., 2008; Han *et al*., 2010a). Additionally, three cryptic *phaC* genes have been identified in the genome of *H. mediterranei* (Han *et al*., 2010b). In addition to the key enzymes, the PHA granule-associated protein of PhaP has also been revealed to be closely related with the PHBV granule formation. Notably, the PhaP protein of *H. mediterranei* shows no homology with bacterial PhaPs (Cai *et al*. 2012).

Significantly, four propionyl-CoA supplying pathways have been revealed to produce propionyl-CoA for PHBV synthesis in *H. mediterranei*, including two novel and major pathways, named citramalate/2-oxobutyrate pathway and 3-hydroxypropionate pathway, respectively (Han *et al*., 2013). This 3-hydroxypropionate pathway, coupling CO2 assimilation with biopolymer accumulation, may have great physiological importance for the carbon assimilation of haloarchaea and also for the carbon cycling in hypersaline ecosystems.

In addition, we have also developed an engineered *H. mediterranei* strain with an increase of approximately 20% in PHBV production (Zhao *et al*., 2013). Therefore, based on our elucidated PHBV biosynthesis pathway and its regulation, *H. mediterranei* has showed great potential to be engineered as a promising archaeal cell factory for future industrial production of PHBV.

Key words: haloarchaea, carbon metabolism, bioplastic production

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